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The link between autophagy and secretion: a story of multitasking proteins

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ABSTRACT The secretory and autophagy pathways can be thought of as the biosynthetic (i.e., anabolic) and degradative (i.e., catabolic) branches of the endomembrane system. In analogy to anabolic and catabolic pathways in metabolism, there is mounting evidence that the secretory and autophagy pathways are intimately linked and that certain regulatory elements are shared between them. Here we highlight the parallels and points of intersection between these two evolutionarily highly conserved and fundamental endomembrane systems. The intersection of these pathways may play an important role in remodeling membranes during cellular stress.

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INTRODUCTION

The term autophagy (“auto”: self; “phagy”: eating) was coined by Christian De Duve, who noted that cells are able to “eat” their own cytoplasmic contents by delivering them to lysosomes (De Duve and Wattiaux, 1966). Autophagy is an evolutionarily conserved process that is responsible for the degradation of superfluous or damaged proteins and organelles. Macroautophagy (hereafter referred to as autophagy) is induced by starvation. Superfluous cellular contents are sequestered in de novo–formed double-membrane-bound vesicles called autophagosomes, which mature through fusion with endomembrane vesicles and lysosomes. Ultimately, the cargo within autophagolysosomes is degraded, and metabolic intermediates are recycled. Because autophagy fulfills an important homeostatic function, it is not surprising that it plays an important role during development and that disruption of this process contributes to the pathogenesis of immune disorders, cancer, and neurodegeneration.

The basic machinery includes >30 autophagy-related (ATG) genes that govern the biogenesis and maturation of autophagosomes. Although these have been best characterized through elegant genetic, biochemical, and cell biological studies in yeast, our understanding of autophagy regulation in mammalian cells is rapidly

emerging (Mehrpour et al., 2010). Various stimuli, including nutrients, energy, and/or growth factor deprivation, activate the autophagy-inducing kinase, called Atg1 in yeast and ULK1/ULK2 in mammals, at the site of autophagosome formation (Mehrpour et al., 2010). Although there are examples of ULK1/2-independent autophagy (Cheong et al., 2011; Lin and Hurley, 2016), ULK/Atg1 forms a conserved complex with ATG13 and FIP200/Atg17 and regulates multiple steps in the canonical autophagy pathway. ULK1/2 phosphorylates multiple components of the autophagy-inducing class III phosphoinositide 3 kinase (PI3K)-VPS34 complex, leading to the generation of phosphatidylinositol 3-phosphate (PI3P), the recruitment of PI3P-binding proteins, and autophagosome membrane nucleation (Russell et al., 2013; Park et al., 2016). ULK/Atg1 also promotes membrane recycling from various sources, including the *trans*-Golgi and recycling endosomes, via the multi-pass transmembrane protein Atg9 (Young et al., 2006; Yamamoto et al., 2012). Subsequently additional autophagy-related proteins, including components of the ubiquitin-like conjugation pathways that lead to lipidation of Atg8, regulate the elongation and closure of autophagosomes.

The process of transporting cargo-containing vesicles is not unique to autophagy and is also an important feature of the secretory pathway, which is responsible for the biogenesis and synthesis of one-third of the cellular proteome. After synthesis and quality control in the endoplasmic reticulum (ER), secretory proteins are exported via coat protein complex II (COPII)–coated carriers that form on ribosome-free regions of the rough ER called ER exit sites (ERES). The COPII coat consists of the Sec23-Sec24 dimer and a Sec13-Sec31 tetrameric complex (Zanetti et al., 2011). In yeast, COPII vesicles fuse directly with the Golgi, but in mammalian cells, COPII vesicles transport cargo to the Golgi via the ER–Golgi

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Abbreviations used: ATG, autophagy-related gene; ER, endoplasmic reticulum; ERES, ER exit sites; ERGIC, ER–Golgi intermediate compartment; PAS, pre-autophagosomal structure.

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intermediate compartment (ERGIC). The bidirectional cycle of transport in the mammalian ER-Golgi system is completed by another class of coated vesicles, COPI, that transport proteins from the ERGIC to the ER.

COMPONENTS OF THE SECRETORY PATHWAY AS A MEMBRANE SOURCE FOR AUTOPHAGY

In yeast, autophagosome assembly is restricted to a discrete site that is located in close proximity to the vacuole (pre-autophagosomal structure [PAS]), whereas in mammals, autophagosome formation is initiated at multiple PI3P-enriched cup-like subdomains of the ER (Axe *et al.*, 2008). ERES reside adjacent to the growing edge of the autophagosomal membrane (Graef *et al.*, 2013; Suzuki *et al.* 2013). In addition, morphological studies revealed contact sites between nascent autophagosomes and ERES, mitochondria, the Golgi, plasma membrane, recycling endosomes, and late endosomes or lysosomes (Lamb *et al.*, 2013; Biazik *et al.*, 2015). The functional significance of many of these contact sites, however, has not been explored, and it is not clear whether these sites are transferring membrane or other components. In support of the importance of membrane exchange is the growing evidence that shuttling of Atg9 between the Golgi and other reservoirs to autophagosomes plays an important role in recycling membranes from these various sources upon autophagy induction (Webber *et al.*, 2007; Yamamoto *et al.*, 2012). There may also be differences in the role of these contacts among different species or indirect consequences of disrupting contact sites. For example, although ER-mitochondria contact sites in mammalian cells were reported to play a role in an early stage of autophagosome formation (Hamasaki *et al.*, 2013), these findings have not been corroborated in yeast (Graef *et al.*, 2013). It is not clear whether this represents a fundamental difference between autophagosome biogenesis in yeast and mammals or the proximity between ERES and ER-mitochondria contact sites in higher eukaryotes has added confusion to the interpretation of the data (Tan *et al.*, 2013).

Nevertheless, the ER is a primary site for autophagosome biogenesis in both yeast and mammals (Graef *et al.*, 2013; Lamb *et al.*, 2013; Suzuki *et al.* 2013; Sanchez-Wandelmer *et al.*, 2015). Proteomics has identified interactions between constituents of ERES and the Atg machinery in yeast (Graef *et al.*, 2013). In mammals, superresolution microscopy has revealed that ULK1/ATG13-positive structures emerge from regions where ATG9-positive vesicles align with the ERGIC (Karanasios *et al.*, 2016). The formation of these pre-autophagosomal structures requires COPII and COPI function (Karanasios *et al.*, 2016). There is also evidence that ERGIC membranes support LC3/Atg8 lipidation, a key step in autophagosome formation, *in vitro* (Ge *et al.*, 2013). Thus, whereas certain secretory compartments (e.g., Golgi, endosomes) may contribute membrane to autophagosomes via ATG9, early compartments of the secretory pathway (i.e., ERES and ERGIC) have a more direct role in the biogenesis of nascent autophagosomes.

MULTITASKING PROTEINS IN SECRETION AND AUTOPHAGY

Role of COPII components in the secretion-autophagy cross-talk

ERES may be a preferred site for autophagosome biogenesis because of the curved membranes that are available at ERES. Alternatively, it is possible that autophagy and the secretory pathway share common machinery that resides at ERES. The first indication that core components of the ER export machinery regulate autophagy came from the observation that yeast strains with genetic defects in certain COPII components or their regulators are unable to generate

autophagosomes (Ishihara *et al.*, 2001). More recently, several proteins, including Sar1, Ypt1/Rab1, and the CK1 kinase Hrr25, which phosphorylates the COPII coat, have been implicated in autophagy (Zoppino *et al.*, 2010; Wang *et al.*, 2015; Davis *et al.*, 2016). Inhibition of the GTPase Sar1, which interacts with its membrane-bound exchange factor Sec 12 and initiates COPII vesicle transport, impairs autophagy in yeast and mammalian cells (Zoppino *et al.*, 2010; Graef *et al.*, 2013), implying an evolutionarily conserved dependence of autophagy on ER export and/or COPII components. In support of the former, treating mammalian cells with the ER-export inhibitor FLI-06 (Krämer *et al.*, 2013) reduces the number of autophagosomes under starvation conditions (Karanasios *et al.*, 2016). Moreover, a recent study in yeast demonstrated that ER export of the ER-localized Qa/t-SNARE Ufe1 is induced in response to starvation and that Ufe1 is targeted to sites containing Atg8 and Atg9, where it contributes to autophagosome formation (Lemus *et al.*, 2016).

The COPII machinery may have a role beyond regulating the trafficking of autophagy regulators and instead may be redirected to autophagy in response to stress. For example, a recent study indicated that phosphorylation of three amino acids on the membrane-distal surface of the COPII cargo adapter Sec24 enhances the interaction of Sec24 with Atg9 at the PAS (Davis *et al.*, 2016; Figure 1). Although phosphorylation of the distal sites on Sec24 is needed to increase autophagosome number, which facilitates cellular homeostasis during starvation, it is not required for ER-to-Golgi transport (Davis *et al.*, 2016). The Sec24 phosphorylation sites are conserved in mammalian SEC24A, and the highly conserved kinase Hrr25 is one of the kinases that phosphorylate this site. The redirection of COPII vesicle machinery for autophagy has also been documented in mammals. Studies demonstrate that the induction of autophagy results in class III PI3 kinase-dependent relocation of ERES components to the ERGIC (Ge *et al.*, 2014).

TECPR2 as a bridge between the autophagy and secretory machinery

Another example of a multitasking regulator is tectonin β -propeller-containing protein 2 (TECPR2), a protein implicated in neurodegenerative diseases (Oz-Levi *et al.*, 2012). TECPR2 binds to ATG8 (LC3 in mammals) and positively regulates autophagy (Behrends *et al.*, 2010; Oz-Levi *et al.*, 2012; Figure 1). ATG8 is a small, ubiquitin-like molecule that is conjugated to phosphatidylethanolamine in the growing autophagosome membrane through a cascade involving other autophagy proteins (Suzuki *et al.*, 2001; Ichimura *et al.*, 2004). Recently TECPR2 was found to interact with the COPII component SEC24D and to regulate the number of ERES in an LC3C-dependent manner (Stadel *et al.*, 2015). The mechanism by which TECPR2 switches from being an ERES regulator to an autophagy regulator remains to be determined.

Rab1 and its dual role in autophagy and secretion

Rab GTPases regulate all membrane-bound intracellular trafficking pathways (Zhen and Stenmark, 2015). Therefore it is not surprising that certain Rabs (Ypt in yeast) regulate both autophagy and secretion. For example, Ypt1 activation on COPII vesicles leads to the recruitment of Usa1, which tethers the vesicles to the Golgi (Cao *et al.*, 1998). Active Ypt1 is also required for starvation-induced autophagy and provides a means of targeting autophagy (i.e., ULK/Atg1) components to the PAS (Wang *et al.*, 2013; Figure 1). Given the degree of evolutionary conservation in this class of proteins, it is perhaps not surprising that mammalian Ypt1 (Rab1) and Trs85, a component of the multimeric guanine nucleotide exchange factor (TRAPPIII) that activates Ypt1/Rab1, have been implicated in the

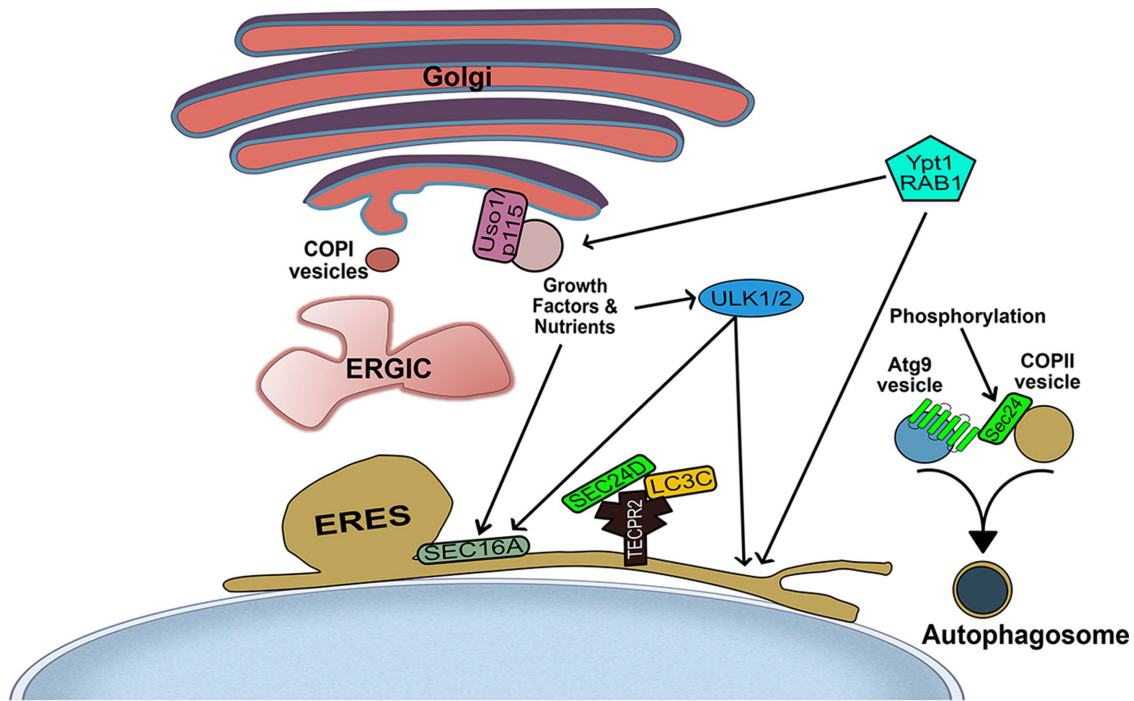


FIGURE 1: Schematic representations of selected modes of cross-talk between autophagy and the secretory pathway. Right, the small GTPase Ypt1/Rab1 regulates the tethering of ER-derived, COPII-coated vesicles to the Golgi in rich medium. During starvation, however, Sec24 is phosphorylated, which promotes the fusion of COPII vesicles with Atg9 vesicles to regulate autophagosome abundance. Middle, growth factors and nutrients signal to the ERES regulator SEC16A. The autophagy-initiating kinases ULK1/2 are also activated by nutrient signaling and phosphorylate SEC16A. Finally (middle, bottom), the autophagy-regulating protein TECPR2 regulates COPII carrier biogenesis in a manner that depends on LC3C, thereby bridging secretion and autophagy.

regulation of autophagy (Behrends *et al.*, 2010; Lynch-Day *et al.*, 2010; Zoppino *et al.*, 2010; Wang *et al.*, 2013).

ULK1/2 are multitasking kinases that regulate autophagy and secretion

ULK1/2, known for their role in the induction and regulation of autophagy (Chan and Tooze, 2009), were recently shown to play a role in ER–Golgi traffic during normal growth (Joo *et al.*, 2016). ULK1/2 localize to ERES and phosphorylate SEC16A (Figure 1), a scaffold protein that facilitates the biogenesis and maintenance of ERES (Sprangers and Rabouille, 2015). Of interest, the phosphorylation of SEC16A by ULK1/2 is required for the trafficking of specific cargo from the ER to the Golgi (Joo *et al.*, 2016). Because SEC16A is a target of several kinases, its posttranslational modification may provide a means of fine-tuning the regulation of COPII transport with the availability of growth factors and nutrients (Farhan *et al.*, 2010; Zacharogianni *et al.*, 2011; Tillmann *et al.*, 2015). Although inhibition of COPII transport impairs starvation-induced autophagy (Ishihara *et al.*, 2001; Zoppino *et al.*, 2010; Graef *et al.*, 2013), additional studies are required to determine whether SEC16A phosphorylation by ULK1/2 is required for autophagy and to precisely elucidate how the multitasking function of SEC16A is orchestrated in the context of secretion and autophagy.

UVRAG and its dual role in autophagy and retrograde COPI transport

In the absence of cellular stress, the PI3P-binding protein UVRAG localizes to the ER, where it assembles into a RINT-1–containing tethering complex that regulates the arrival of COPI vesicles involved in Golgi-to-ER retrograde trafficking (He *et al.*, 2013). On the induction

of autophagy, UVRAG dissociates from the RINT-1 complex and associates with the class III PI3K complex to mediate the mobilization of ATG9 from the Golgi (He *et al.*, 2013; Figure 1). UVRAG also regulates autophagosome maturation by binding to the class C Vps complex (Liang *et al.*, 2008). Thus UVRAG is a multitasking protein whose function is altered in response to cellular stress.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Connections between secretory traffic and autophagy are rapidly emerging, and, as discussed, we have already identified several “multitasking” proteins that participate in both pathways. Yet, the extent of cross-talk between these pathways remains to be determined, and there are many unanswered questions. For example, how do autophagy-inducing stimuli affect the secretory pathway with respect to structure, function, and molecular composition? What are the signals and molecular events that allow proteins to switch tasks in response to changes in metabolism? Do basal autophagy and starvation-induced autophagy have different dependences on ER export?

Although a few systematic screens have been performed on either the autophagy or the secretory pathway, we are far from a systematic understanding of the cross-talk between these two pathways (Farhan, 2015). A combination of RNA interference or clustered regularly interspaced short palindromic repeats (CRISPR) screening with chemical biology will be required to obtain an integrative view of the coregulation of secretory trafficking and autophagy. This is very important because alterations of the secretory pathway or autophagy have been implicated in several human diseases. For instance, defects in trafficking to and from the Golgi apparatus and alterations in Golgi structure are common in neurodegenerative

disorders and may be direct or indirect consequences of certain disease-causing mutations (Joshi *et al.*, 2015). Similarly, alterations in the autophagy-lysosomal pathway have been implicated in pathogenesis of neurodegenerative diseases (Nixon, 2013). The role of the cross-talk between autophagy and secretion in diseases is an exciting topic for future studies.

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